Claims

1. A method for screening a protein interactive with PPARγ in a ligand-dependent manner, utilizing a yeast two-hybrid system in the presence of a PPAR ligand with a high potency of triggering the action ameliorating glucose metabolism, wherein a polynucleotide encoding a region containing at least the position 204 to position 505 of the PPARγ protein represented by SEQ ID NO: 2 is used as bait and a cDNA library is used as prey.

2. A method for screening a protein interactive with PPARγ in a ligand-dependent manner, utilizing a yeast two-hybrid system in the presence of a PPAR ligand with a high potency of triggering edema, wherein a polynucleotide encoding a region containing at least the position 204 to position 505 of the PPARγ protein represented by SEQ ID NO: 2 is used as bait and a cDNA library is used as prey.

3. A cell transformed by i) a polynucleotide encoding a polypeptide consisting of an amino acid sequence of SEQ ID NO: 4 or a polynucleotide encoding a polypeptide comprising an amino acid sequence represented by SEQ ID NO: 4 wherein 1 to 10 amino acids therein are deleted, substituted and/or inserted and also interacting with PPAR in a ligand-dependent manner, ii) a gene encoding a fusion

protein comprising at least the ligand binding region of the PPAR protein represented by SEQ ID NO: 2 or 6 and the DNA binding region of a transcription factor, and iii) a reporter gene fused to a response element to which said DNA binding region of the transcription factor is capable of binding; or a cell transformed by i) a polynucleotide encoding a polypeptide consisting of an amino acid sequence of SEQ ID NO: 4 or a polynucleotide encoding a polypeptide comprising an amino acid sequence represented by SEQ ID NO: 4 wherein 1 to 10 amino acids therein are deleted, substituted and/or inserted and additionally interacting with PPAR in a ligand-dependent manner and ii) a reporter gene fused to a response element to which the DNA binding region of the PPAR protein represented by SEQ ID NO: 2 or 6 is capable of binding, said cell expressing a) a polypeptide consisting of an amino acid sequence of SEQ ID NO: 4 or a polypeptide comprising an amino acid sequence represented by SEQ ID NO: 4 wherein 1 to 10 amino acids therein are deleted, substituted and/or inserted and interacting with PPAR in a ligand-dependent manner and b) the PPAR protein represented by SEQ ID NO: 2 or 6.

4. A cell transformed by i) a polynucleotide encoding a polypeptide consisting of an amino acid sequence of SEQ ID NO: 8 or a polynucleotide encoding a polypeptide comprising an amino acid sequence represented by SEQ ID NO:

8 wherein 1 to 10 amino acids therein are deleted, substituted and/or inserted and additionally interacting with PPAR in a ligand-dependent manner, ii) a gene encoding a fusion protein comprising at least the ligand binding region of the PPAR protein represented by SEQ ID NO: 2 or 6 and the DNA binding region of a transcription factor, and iii) a reporter gene fused to a response element to which said DNA binding region of the transcription factor is capable of binding,; or a cell transformed by i) a polynucleotide encoding a polypeptide consisting of an amino acid sequence of SEQ ID NO: 8 or a polynucleotide encoding a polypeptide comprising an amino acid sequence represented by SEQ ID NO: 8 wherein 1 to 10 amino acids therein are deleted, substituted and/or inserted and additionally interacting with PPAR in a ligand-dependent manner and ii) a reporter gene fused to a response element to which the PPAR protein represented by SEQ ID NO: 2 or 6 is capable of binding, said cell expressing a) a polypeptide consisting of an amino acid sequence of SEQ ID NO: 8 or a polypeptide comprising an amino acid sequence represented by SEQ ID NO: 8 wherein 1 to 10 amino acids therein are deleted, substituted and/or inserted and interacting with PPAR in a ligand-dependent manner, and b) the PPAR protein represented by SEQ ID NO: 2 or 6.

5. A cell according to claim 3 or 4, wherein the transcription factor is the GAL4 protein of yeast.

6. A cell according to claim 3 or 4, wherein the reporter gene is luciferase gene.

7. A method for detecting whether or not a test substance promotes the action of ameliorating glucose metabolism via PPAR, comprising i) a step of allowing a cell according to claim 3, a PPAR ligand and a test substance in contact with each other, and ii) a step of analyzing the change of the ligand-dependent interaction or the change of the transcriptional activity induced by ligand-activated PPAR, using the expression of a reporter gene as a marker.

8. A method for screening a drug ameliorating insulin resistance, comprising i) a step of allowing a cell according to claim 3, a PPAR ligand and a test substance in contact with each other, and ii) a step of analyzing the change of the ligand-dependent interaction or the change of the transcriptional activity induced by ligand-activated PPAR, using the expression of a reporter gene as a marker.

9. A method for screening according to claim 8, wherein the drug ameliorating insulin resistance is a drug ameliorating glucose metabolism.

10. A method for detecting whether or not a test substance promotes the activity triggering edema via PPAR, comprising i) a step of allowing a test substance in contact with a cell according to claim 4, and ii) a step of analyzing the change of the interaction due to the test substance or the change of the transcriptional activity induced via PPAR due to the test substance using the expression of a reporter gene as a marker.

11. A method for screening a drug ameliorating insulin resistance with no activity of triggering edema, comprising i) a step of allowing a test substance in contact with a cell according to claim 4, ii) a step of analyzing the change of the interaction due to the test substance or the change of the transcriptional activity induced via PPAR due to the test substance, using the expression of a reporter gene as a marker; and iii) a step of selecting a test substance not enhancing the reporter activity.

12. A method for screening according to claim 11, wherein the drug ameliorating insulin resistance is a drug ameliorating glucose metabolism.

13. A cell transformed by i) a polynucleotide encoding a polypeptide consisting of an amino acid sequence of SEQ ID NO: 17 or a polynucleotide encoding a polypeptide comprising an amino acid sequence represented by SEQ ID NO: 17 wherein 1 to 10 amino acids therein are deleted, substituted and/or inserted and also interacting with PPAR in a ligand-dependent manner, ii) a gene encoding a fusion protein comprising at least the ligand binding region of the PPAR protein represented by SEQ ID NO: 2 or 6 and the DNA binding region of a transcription factor, and iii) a reporter gene fused to a response element to which said DNA binding region of the transcription factor is capable of binding; or

a cell transformed by i) a polynucleotide encoding a polypeptide consisting of an amino acid sequence of SEQ ID NO: 17 or a polynucleotide encoding a polypeptide comprising an amino acid sequence represented by SEQ ID NO: 17 wherein 1 to 10 amino acids therein are deleted, substituted and/or inserted and additionally interacting with PPAR in a ligand-dependent manner and ii) a reporter gene fused to a response element to which the PPAR protein represented by SEQ ID NO: 2 or 6 is capable of binding,

said cell expressing a) a polypeptide consisting of an amino acid sequence of SEQ ID NO: 17 or a polypeptide comprising an amino acid sequence represented by SEQ ID NO: 17 wherein 1 to 10 amino acids therein are deleted, substituted and/or inserted and interacting with PPAR in a ligand-dependent manner, and b) the PPAR protein represented by SEQ ID NO: 2 or 6.

14. A method for detecting whether or not a test substance promotes the action of ameliorating glucose metabolism via PPAR, comprising i) a step of allowing a test substance in contact with a cell according to claim 13, and ii) a step of analyzing the change of the interaction due to the test substance or the change of the transcriptional activity induced via PPAR due to the test substance, using the expression of a reporter gene as a marker.

15. A method for screening a drug ameliorating insulin resistance, comprising i) a step of allowing a cell according to claim 13 in contact with a test substance, and ii) a step of analyzing the change of the interaction due to the test substance or the change of the transcriptional activity induced via PPAR due to the test substance, using the expression of a reporter gene as a marker.

16. A method for screening according to claim 15, wherein the drug ameliorating insulin resistance is a drug ameliorating glucose metabolism.

17. A method for screening a drug ameliorating insulin resistance, comprising i) a step of allowing a test substance in contact with a cell transformed with a reporter gene fused to a polynucleotide consisting of a nucleotide sequence of SEQ ID NO: 26 or a polynucleotide comprising a nucleotide sequence represented by SEQ ID NO: 26 wherein 1 to 10 bases therein are deleted, substituted and/or inserted and also having a transcription promoter activity, and ii) a step of analyzing the change of the activity for transcriptional induction due to the test substance, using the expression of a reporter gene as a marker.

18. A method for screening according to claim 17, wherein the reporter gene is the luciferase gene.

19. A method for producing a pharmaceutical composition for ameliorating insulin resistance, comprising a screening step using a screening method according to claim 8, 11, 15 and/or 17 and a formulation step using a substance obtained by the screening.